Roger Tsien: Bringing color to cell biology

A passion for chemistry, color, and conversations with cells is what drives Roger Tsien.

Seeing what cells are doing is easier in color. Roger Tsien has made life easier for cell biologists everywhere by designing and synthesizing colorful molecular tools for visualizing cell components and cell activity. His early work focused on creating fluorescent dyes for monitoring cellular calcium (1, 2), and he has used similar techniques to monitor sodium and cyclic AMP (3, 4). Tsien has also exploited and improved fluorescence resonance energy transfer (FRET) for detecting interactions between two fluorescently labeled molecules (5, 6).

The work for which Tsien is arguably most famous, though, is his genetically engineered wavelength variants of green fluorescent protein (5, 7) and, more recently, red fluorescent protein (8). This work has provided the cell biology community with an entirely new palette of fluorescent tags for labeling proteins of interest and has also brought Tsien a number of prestigious awards.

Talking with Tsien in a recent interview, it was clear he has a colorful character to match his colorful career.

CHILDHOOD CHEMISTRY

What sparked your enthusiasm for science?

It's in my family. My father was an engineer, my mother was a nurse, which is pretty much as close as a Chinese woman could get to science during WWII, and my oldest brother, Dick, is an eminent neurobiologist at Stanford.

I have lots of engineers amongst my relatives, on both my mother's and father's side. My father's cousin was quite a famous engineer (H.S. Tsien). He was the head of the ballistic missile program for the People's Republic. It's actually a strange tale. He was at Cal Tech as an aeronautical engineer, but then got in trouble in the 1950s because he had some communist links in his past. He was put under house arrest and then deported. Back in China, he started up the ballistics program, I guess partly because he was mighty pissed off at the US!

So, science and engineering are in your blood.

Yes, and I remember my first interest in science was actually in chemistry. My parents had bought me a chemistry set, but I found it boring because the experiments were so safe. But then I found a book in the school library that told you how to make a bright purple solution turn bright green, just by passing it through a funnel of filter paper. Amazingly, it worked when I tried it, which I thought was pretty cool. So I decided chemistry was more fun than chemistry sets let on. That was in elementary school. By high school, I had started to accumulate stuff in the basement at home. One time, my brother and I surreptitiously made gunpowder.

> "I realized that chemistry could actually be quite interesting if you had the right motivation."

Were you setting up your own ballistics program?

We thought we could set off a controlled explosion, but it was just slightly out of control—we set fire to the ping-pong table! We managed to put it out, but there was a good deal of smoke, and my parents got a little bit alarmed.

After that incident, I tended to shift to the outdoor patio, which was made of concrete. That was a safer place. It was effectively like working in a fume hood.

How did you go from bombs to biology?

I thought I was going to be a chemist. I was good at it in high school and was awarded the Westinghouse Science Prize for an NSF-funded chemistry project I did in the summer of my junior year, 1967.

Roger Tsien

But I went off to Harvard and found that I hated the way chemistry was taught there. I resolved that chemistry was really boring. I couldn't see the point of organic chemistry. I couldn't stand it anymore. I started drifting around, looking for things, and eventually the thing that most attracted me was neurobiology.

COLORFUL SOLUTIONS

After Harvard, you studied for a Ph.D. in England with Richard Adrian. But he wasn't a neuroscientist, was he?

No. When it was time to look for a graduate school, I received a Marshall Scholarship for Cambridge. I heard from the Marshall Commission that I had been assigned to a Dr. R.H. Adrian as my Ph.D. supervisor, but I had never met him.

As it turned out, my older brother, Dick, who had just come back from Oxford, knew Richard Adrian. I asked him, "Who's this Richard Adrian?" He said, "Oh, well, he's a very eminent muscle electrophysiologist." I said, "Muscle? You're kidding me; I want to work on the brain. Muscle is a backwater!"

Dick said, "Don't worry, Richard Adrian is a true British gentleman. He'll let you work on whatever you want." So,

I thought, "I'll see how it goes. I won't immediately write a letter of protest." That was maybe January or February '72 before I had finished at Harvard.

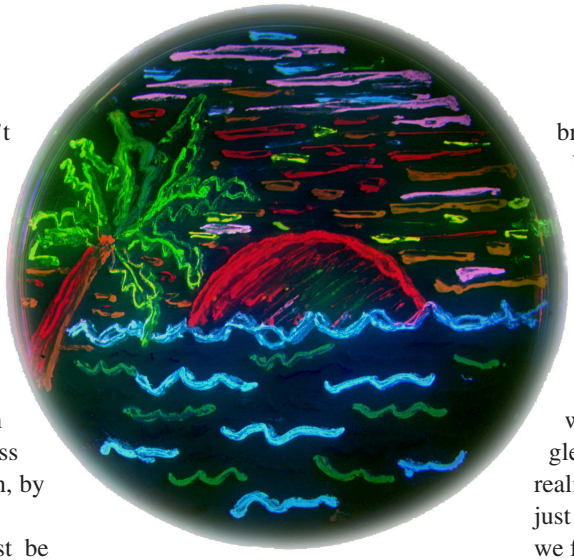
In October, I went to Cambridge University. I was sitting in Churchill College Refectory, and this distinguished gentleman in a tweed jacket sat down across from me and asked me in a very upper-class British accent, "Are you Roger Tsien, by any chance?"

I guessed correctly that he must be Richard Adrian, who had come looking for me. Within about five minutes, he asked, "Is it true that you think muscle is a backwater?"

That's how I got started with my Ph.D. supervisor!

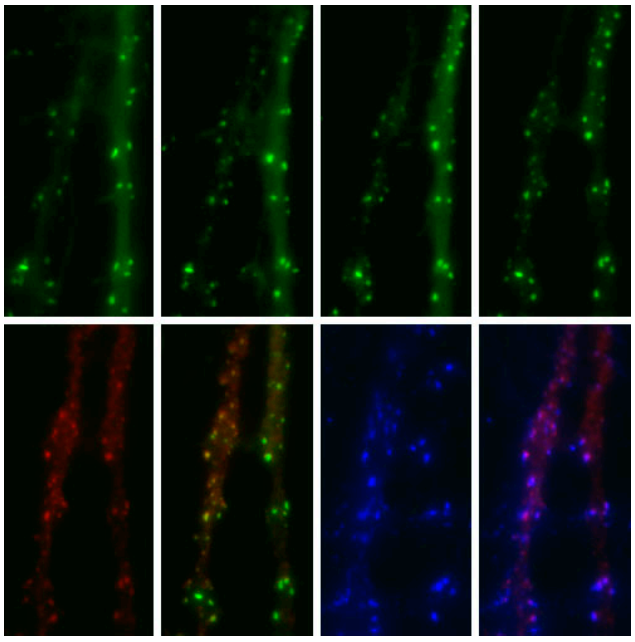
Oh dear. But, Dick was right. Adrian did let you work on your neuroscience project. Tell me about that.

Neurobiology at the time was dominated by single-neuron recordings. People would drill a hole in the skull, drop an electrode blindly into the cortex, and start recording. It's like ice fishing, and after you've caught a couple hundred fish over several years, you write a Ph.D. describing in detail the classification of the fish that you pulled up from beneath the ice.



Bacteria expressing different genetically engineered fluorescent proteins take a vacation in a petri dish.

People would write for their theses, "I recorded 300 neurons from this region of the brain, and this was their firing pattern, and this is how they responded to this, that, or the other stimulus." I didn't like that. I wanted some means by which one could see the activity of the brain in a more parallel fashion. The brain is made up of billions of neurons, and listening to them one at a time has limitations. It would be better, I thought, to see networks of neurons signaling to each other. That immediately implied imaging.



Tsien detects protein turnover in neuronal synapses (blue) by distinguishing new (red) and old (green) scaffold proteins.

How did the project unfold?

To begin with, I wanted to image membrane potential. This was difficult because any dye you make should be just in the plasma membrane and not in the internal membranes, and also because it wasn't clear how a dye would sense the voltage. Larry Cohen and his collaborators at Yale were randomly testing lots of photographic dyes for sensitivity. They had some success, but I attempted to be clever and specifically design just a few. I was not successful at the time. I struggled with that for years and eventually realized that, for the time being, it was just too hard, though two decades later we found a way.

Targeting calcium, however, was an enormously simpler solution to the problem of following neuronal activity. Action potentials almost always lead to major calcium influxes somewhere in the neuron. Chemically speaking, a lot was known about calcium, but it was ignored by biologists. Meanwhile, chemists were unaware of the biological importance of calcium signals. That gave me an angle, because there was almost no competition. My crummy chemistry on a chemically easier problem produced a unique solution.

"I've always liked pretty colors; it's valuable to get some degree of gut pleasure out of what you're doing."

Strangely enough, this is when I decided chemistry wasn't so bad. I realized that chemistry could actually be quite interesting if you had the right motivation. That's what was missing before. Chemistry for its own sake didn't interest me, but biology has interesting problems, for which chemistry can provide solutions.

Most biologists hate or fear chemistry. If a biological problem needed a chemical solution that you couldn't just buy, nobody expected you to do it. It was assumed that chemistry is impossible for outsiders. That view is gradually changing. But back when we started, it

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gave us a tremendous opportunity, because being able to do any chemistry was like being the one-eyed man in the kingdom of the blind.

That's been a fantastic niche for you throughout your entire career.

It has been very useful. For a long time, it kept the competition out. We could be very simple minded as chemists, but since hardly any chemists knew of the problems, and few biologists could do any chemistry, we had the problem pretty much to ourselves!

After making dyes for calcium you moved on to engineering a multicolored palette of fluorescent proteins. Your career has been filled with pretty colors.

I've always liked pretty colors. I tell students it's valuable to get some degree of gut pleasure out of what you're doing, to keep you going. Because, yes, when experiments work, there's nothing like that thrill, but that happens once in a while, and you can go for long, long periods where nothing works.

Enjoying pretty colors is one of my ways to keep going. It may be why I wound up doing this type of science. When all you are doing is looking at colorless solutions being pipetted from one tube to another, and visualizing the results on a gel by radioactivity much later, I find that very dry.

I much prefer experiments where there are pretty colors, and real activity, where the cells can talk back to you while they are alive. It makes it possible to design the experiments based on the conversation you're having with them.

FLUORESCENT FUTURE

Which cells are you having a conversation with these days?

We're imaging neurons to look at protein turnover at synapses, and we're also imaging tumors inside animals.

Have you designed new tools for imaging these?

Yes. For the neurons, we use a special protease from hepatitis C virus. We fuse an epitope tag to our protease and then fuse the protease to our protein of interest. As the protein comes off the ribosome, the protease immediately cuts itself and the epitope tag off, leaving the host protein unmarked.

That happens throughout the life of the animal until we deliver a drug that specifically blocks this protease. From the time you administer the drug onward, all new copies of the protein remain intact and tagged.

Immunofluorescence against the tag will then light up synapses with new protein turnover. Being able to distinguish between newly made and old proteins should give us a more direct attack on the question of where brains store, for example, short-term and long-term memory.

How do you image the tumors?

We have capitalized on peptide sequences that are known to be taken up into cells, called cell-penetrating peptides (CPPs). If you tie the positively charged CPPs to negative charges, you get something that's fairly biologically inert and that won't get taken into cells unless the linker between the plus and minus ends is cut. Cancers express particular enzymes that they use to cut through the extracellular matrix to be invasive. When our inert fluorescently labeled CPPs reach the tumor, the tumor cells cut through the linker and thus unleash and take up the CPP. We're making the tumor cells take up fluorescence, but it could be used for other types of imaging such as magnetic resonance imaging—and perhaps eventually even for putting in drugs to kill the cancer.

Sounds like you're moving into a more clinical phase of your career.

I'm getting fussier and fussier about the importance of the problem I want to work on. Imaging cancer is pretty important. My father died from cancer, and so did Richard Adrian, my Ph.D. supervisor. It would be nice to contribute something in that area.

I probably only have time for one more phase in my career. The neuronal and the cancer projects are my attempt to do something different. We're reinventing ourselves, starting again in fields where we're not the experts anymore. Who knows how this phase is going to work out?

I have to say, that's one thing that getting some prizes for the GFP work was good for. I'd rather not just use the prizes to congratulate myself. It feels more like they've given me a license to go and try something else, like, "You've been there, you've done that, now do something better." JCB

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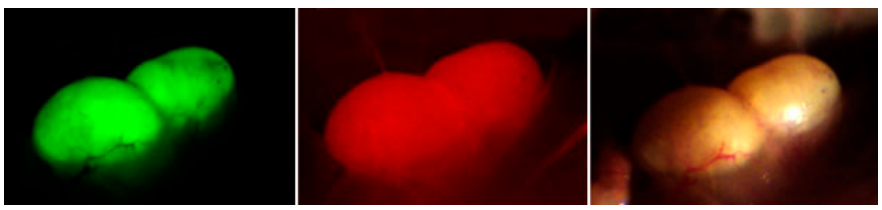

Coloring-in cancers: Tsien's imaging tricks might illuminate the way for drug delivery.